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959,853

PATENT SPECIFICATION

NO DRAWINGS

959,853

Inventors: BERNDT OLOF HARALD SJOBERG and BERTIL AKE EKSTROM

Date of filing Complete Specification (under Section 3 (3) of the Patents Act 1949): Feb. 4, 1963.

Application Date: Feb. 28, 1962. No. 7751/62.

Application Date: Feb. 28, 1962. No. 7752/62.

Complete Specification Published: June 3, 1964.

Index at acceptance:—C2 A(1C2A, 1C2C, 2C1); C2 C2D27; C3 S(2, 3A, 5, 6, 7B, 7D)

International Classification:—C 07 d (C 07 f)

COMPLETE SPECIFICATION

Penicillins

ERRATUM

SPECIFICATION No. 959,853
Amendment No. 1

Page 4, line 65, *for* "-2:6-" *read* "-2:5-"

THE PATENT OFFICE
2nd September 1966

SPECIFICATION NO. 959,853

BY a direction given under Section 17 (1) of the Patents Act 1949 this application proceeded in the name of BEECHAM GROUP LIMITED, a British Company, of Beecham House, Great West Road, Brentford, Middlesex.

THE PATENT OFFICE

D 102425/1

hydrogen atom of the alkyl, aryl or aralkyl group which may be substituted, which process comprises reacting a compound of the general formula:

... and non-toxic salts including ... toxic metallic salts such as sodium, potassium, calcium and aluminium, ammonium and substituted ammonium salts, e.g. salts of such

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COMPLETE SPECIFICATION

PATENTS ACT, 1949

SPECIFICATION NO. 959,853

In accordance with the Decision of the Superintending Examiner, acting for the Comptroller-General, dated the 20th day of November 1967 this Specification has been amended under Section 14 in the following manner:-

Page 1, line 43, *after* "protected" *insert* "against taking part in the reaction by being either protonated, included in a cyclic anhydride, such as an oxazolid-2,5-dione, or condensed with an aromatic hydroxy aldehyde to a Schiff base"

Page 1, line 45, *delete* "The amino group in"

Page 1, *delete* lines "46 to 50" inclusive

Page 8, line 10, *after* "protected" *insert* "by being protonated or included in a cyclic anhydride"

Page 8, *after* line 12, *insert* "2. A process as claimed in claim 1, wherein instead of the methods of protection of the amino group therein set forth, the amino group in the acid (III) is protected by being condensed with an aromatic hydroxy aldehyde to a Schiff base."

Page 8, *for* claims "2 to 8" *read* "3 to 9" inclusive

Page 8, line 13, *after* "claim 1" *insert* "or claim 2"

Page 8, lines 25, 26 and 45, *delete* "claim 1 or claim 2" *insert* "any one of claims 1 to 3"

Page 8, lines 34 and 37, *for* "1 to 3" *read* "1 to 4"

Page 8, line 49, *for* "1 to 7" *read* "1 to 8"

Reference has been directed, in pursuance of Section 9, subsection (1) of the Patents Act, 1949, to Patent No. 964,449.

THE PATENT OFFICE,
14th February 1968

D 100786/9

959,853

PATENT SPECIFICATION

NO DRAWINGS

959,853

Inventors: BERNDT OLOF HARALD SJOBERG and BERTIL AKE EKSTROM

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International Classification:—C 07 d (C 07 f)

COMPLETE SPECIFICATION

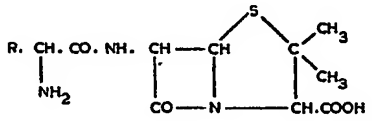
Penicillins

We, BEECHAM RESEARCH LABORATORIES LIMITED, a British Company, of Great West Road, Brentford, Middlesex, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to penicillins and is particularly concerned with a process for the preparation of α-amino-substituted penicillins from reactive silyl derivatives of 6-aminopenicillanic acid.

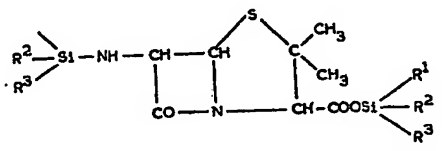
We have now found that penicillins containing free amino groups in the acyl moiety can be prepared from a homogenous solution of a reactive silyl derivative of 6-aminopenicillanic acid, *e.g.* a 6-N-trialkylsilylaminopenicillanic acid trialkylsilyl ester in an anhydrous organic solvent with or without an organic base present. In this way it is possible to obtain such penicillins in yields in excess of those obtained with the conventional methods.

Accordingly, the present invention provides a process for the preparation of penicillins of the general formula:



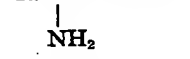
(I)

and non-toxic salts thereof, where R is a hydrogen atom or an alkyl, aryl or aralkyl group which may be substituted, which process comprises reacting a compound of the general formula:



(II)

where R^1, R^2 and R^3 are the same or different and each is an alkyl, aryl or aralkyl group, or a solution thereof, or the crude reaction mixtures which are obtained when 6-aminopenicillanic acid is allowed to react with silylating agents, or solutions of such mixtures, with a reactive derivative of a carboxylic acid of the general formula:



(III)

in which the amino group is protected, and thereafter removing the silyl groups by hydrolysis or alcoholysis. The amino group in III is prevented from taking part in the reaction in that it is either protonated, included in a cyclic anhydride, such as an oxazolid-2:5-dione, or condensed with an aromatic hydroxyaldehyde to a Schiff base.

Examples of reactive derivatives include activated esters, carboxylic acid chlorides or bromides, acid anhydrides or mixed anhydrides with other carboxylic, sulphonic or inorganic acids, or derivatives obtained from a carboxylic acid and a carbodiimide or an N,N¹-carbonyldiimidazole.

The salts are non-toxic salts including non-toxic metallic salts such as sodium, potassium, calcium and aluminium, ammonium and substituted ammonium salts, e.g. salts of such

non-toxic amines as trialkylamines, including triethylamine, procaine, dibenzylamine, N-benzyl - beta - phenethylamine, 1 - ephenamine, N,N¹ - dibenzylethylenediamine, dehydroabietylamine, N,N¹ - bis - dehydroabietylethylenediamine, and other amines which have been used to form salts with benzylpenicillin.

In one form of the present invention penicillins containing free amino groups in the acyl moiety have been prepared in good yields by reacting the silyl derivatives of 6-aminopenicillanic acid with cyclic anhydrides of amino acids, *e.g.* oxazolid 2:5 diones, or with amino acid chloride hydrochlorides.

Organic tertiary bases such as triethylamine, N,N-dimethylaniline, ethylpiperidine, 2,6-lutidine, quinoline or even an excess of the silyl compound can be used as proton acceptors or salt forming agents.

The reactions with the anhydrides are usually performed at low temperatures and under the reaction conditions used the carbamic acids formed are stabilized as salt with the bases. In this way the amino group of the anhydrides cannot react further and unwanted side reactions are avoided. When the temperature is allowed to rise after the coupling reaction is completed, the carbamic acid salt decomposes and the amino group is liberated. By addition of water or an alcohol the trialkylsilyl groups are hydrolysed and the desired penicillin obtained.

In the acylation with amino acid chloride hydrochlorides the base used should not be such as to remove the hydrochloride from the amino acid. After the coupling reaction is completed the hydrochloride is easily split off by addition of water and adjustment of the pH.

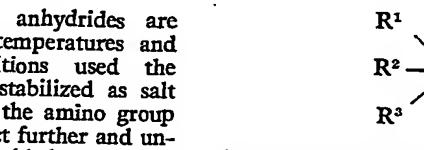
In another form of the invention a Schiff base of an amino acid with an aromatic hydroxyaldehyde, such as 2 - hydroxy - 1-naphthaldehyde or 5-chlorosalicylaldehyde is treated with a chloroformic acid ester in an anhydrous solvent, such as dimethylformamide, acetone, chloroform or tetrahydrofurane at a temperature below 0°C., and whereafter the mixed anhydride obtained is allowed to react *in situ* with a reactive silyl-derivative of 6-amino-penicillanic acid. Afterwards water is added and the mixture is acidified to pH 2 for a short period of time to hydrolyse the silyl groups and the arylidene group.

The removal of the silyl groups is very readily performed by addition of water or an alcohol to the reaction mixture. The silyl groups are thereby immediately transformed into disiloxanes or alkoxysilanes, which are soluble in organic solvents and conveniently removed from the reaction mixture. The easy removal of the protecting groups used according to this invention contrasts markedly with the difficulties experienced with other protecting groups, *e.g.* the carbobenzyloxy group, used in the conventional synthesis of amino penicillins from 6-aminopenicillanic acid.

The products prepared according to the present invention can be isolated and purified by the techniques used with other aminopenicillins.

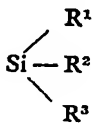
If the acid of the general formula III contains one or more asymmetric centres the corresponding penicillins can exist in diastereoisomeric forms, which are all biologically active. It is to be understood that this invention comprises the diastereoisomers as well as mixtures of them.

The silyl derivatives of 6-aminopenicillanic acid used as starting materials in the process of the present invention are prepared by reacting 6-aminopenicillanic acid or a salt thereof with a compound of the general formula:



(IV)

wherein R^1, R^2 and R^3 have the same meaning as above and X is halogen or the group $N<^{R^4}_{R^5}$ or $S—R^5$ where R^4 is hydrogen or R^1, and R^5 is hydrogen, R^1 or



Thus the 6 - N - trialkylsilylaminopenicillanic acid trialkylsilyl esters can be prepared by treating a salt of 6-aminopenicillanic acid, *e.g.* the sodium, potassium or calcium salt, with a chlorotrialkylsilane in an organic solvent such as benzene, toluene, formamide or dimethylformamide in presence of an organic tertiary base, such as triethylamine, ethylpiperidine or 2,6-lutidine. After stirring for some hours a solution of the silyl compound is obtained which can if desired be used for the synthesis of penicillins. If the solvent is removed *in vacuo* the silyl compound is left as a thick oily residue which may be crystallized.

A further method for preparing the silyl compounds involves heating 6-aminopenicillanic acid with excess of an hexaalkyldisilazane at an elevated temperature whilst continuously removing the liberated base.

A yet further and preferred method of preparing the trialkylsilyl derivatives of 6-aminopenicillanic acid consists in heating a mixture of 6-aminopenicillanic acid with more than two moles of a N-trialkylsilyldialkylamine, such as N-trimethylsilyldiethylamine or N-

trimethylsilyldimethylamine, to a temperature of from 50 to 170°C. so that the dialkylamine, which is formed in the reaction, is distilled off. The reaction is usually completed within one hour and the excess of trialkylsilyldialkylamine is removed *in vacuo* leaving the trialkylsilyl derivative of 6-aminopenicillanic acid as a thick oil which may be crystallized.

The silyl derivatives are soluble in organic solvents as ether, tetrahydrofurane, dioxane, acetone, benzene, toluene, petroleum ether, methylene chloride, chloroform, formamide and dimethylformamide, and also in the trialkylsilyldialkylamines which may be used as reactants. By cooling the solutions in *e.g.* diethyl ether, petroleum ether or N-trimethylsilyl-diethylamine the silyl derivative may crystallize.

The substituted silyl groups in the compounds are easily removed by addition of water or an alcohol such as methanol or ethanol and owing to their sensitivity towards hydroxylating agents they are best prepared under strictly anhydrous conditions.

The following Examples 4 to 12 illustrate the present invention, Examples 1, 2 and 3 describing the preparation of the intermediates.

Example 1

4.3 g. (0.02 mole) of 6-aminopenicillanic acid was mixed with 14.5 g. (0.1 mole) of N-trimethylsilyldiethylamine and heated with stirring, a slow stream of dry nitrogen being passed through the reaction mixture. At 60 to 70°C. a rapid dissolution of the 6-aminopenicillanic acid occurred and at 80°C. a liquid started to distill over. After 20 minutes the temperature was gradually raised to 150°C. and held there for 5 minutes in order to complete the reaction. The total reaction time was 45 minutes. The reaction mixture was then allowed to cool to 80°C., whereafter all volatile components were distilled off, finally *in vacuo*, leaving behind an oily residue.

The residue was evacuated for 1 hour at 0.5 mm Hg giving 6 - N - trimethylsilylaminopenicillanic acid trimethylsilyl ester as a thick brownish oil. (7.1 g. corresponding to a yield of 98.5% of the crude product). The residue was dissolved in 30 ml. of dry petroleum ether (b.p. 40/60°) and a small amount of insoluble products separated by filtration. The trimethylsilyl derivative separated as small colourless crystal needles, which were collected by filtration under rigorously dry conditions. After washing with petroleum ether and drying in a stream of dry nitrogen 5.4 g. of the product was obtained with a melting point of 67 to 68°C.

Analysis: $C_{14}H_{28}N_2O_3SSi_2$ (360.57): % Si
found: 15.4
calculated: 15.56

The compound was characterised through its IR-spectrum, which among others showed absorption bands at 3390, 2970, 1775, 1720, 1270—50 and 840 cm^{-1}, indicating the presence of a NH group, methyl groups, β-lactam linkage, carboxylic ester group and trimethylsilyl groups respectively. The compound was soluble in anhydrous organic solvents such as diethylether, dioxane, tetrahydrofurane, acetone, benzene, toluene, petroleum ether, chloroform, methylenechloride, formamide and dimethylformamide. By addition of water ethanol or methanol to such solutions there was immediately obtained a white precipitate, which dissolved in dilute alkali and could be reprecipitated by addition of acid. The IR-spectrum of this product was in all respects identical with that of 6-aminopenicillanic acid. The recovery of 6-aminopenicillanic acid was nearly quantitative.

Other experiments showed that the reaction with an equally good result could be performed by using 2—5 mol. of the N-trimethylsilyl diethylamine per mol. of 6-aminopenicillanic acid and by using a reaction temperature varying between 70—170°C.

The N-trimethylsilyl diethylamine used in this example was prepared as described by Sauer and Hasek, J.Am.Chem.Soc., *68*, 241 (1946).

Example 2

4.7 g. (0.01 mole) of the calcium salt of 6-aminopenicillanic acid was suspended in 25 ml. of dry benzene; 2.02 g. (0.02 mole) of dry triethylamine and 4.35 g. (0.04 mole) of trimethylchlorosilane were added and the mixture heated with stirring for 3 hours at 60°C. in an atmosphere of dry nitrogen. The reaction mixture was filtered with precautions to exclude moisture and the resulting clear solution was evaporated *in vacuo* giving 2.1 g. (29%) of the 6 - N - trimethylsilylaminopenicillanic acid trimethylsilyl ester as an oily residue. The IR spectrum of this product showed identity with the product obtained in Example 1.

The calcium salt used in this example was obtained by dissolving 6-aminopenicillanic acid with a calculated amount of calcium hydroxide in water and freeze drying the resulting solution. The salt was 96% pure as determined with the hydroxylamine test. Before use it was dried *in vacuo* over phosphorus pentoxide.

By substituting 0.02 mol. of the sodium salt of the 6-aminopenicillanic acid for the calcium salt in this example and following the above procedure, there was obtained 1.8 g. (25%) of the bis-trimethylsilyl derivative of 6-aminopenicillanic acid. The identity with the product from Example 1 was ascertained, by infrared analysis.

Example 3

10.8 g. (0.05 mole) of dried 6-aminopenicillanic acid was mixed with 24.2 g. (0.15 mole) of hexamethyldisilazane and the mixture heated with stirring whilst a slow stream of dry nitrogen was passed therethrough, the reaction commencing at 90°C. with evolution

of ammonia. After 3 hours at 120°C. the reaction mixture was allowed to cool to room temperature and 25 ml. of dry ether added. The mixture was then filtered under moisture-free conditions and the resulting clear solution concentrated *in vacuo* to give 11.6 g. corresponding to a yield of 64.5% of 6-N-trimethylsilylamino penicillanic acid trimethylsilyl ester as a slightly yellow thick oil. This product was shown by infrared analysis to be identical with the product obtained in Example 1. The hexamethyldisilazane used in this example was prepared according to Sauer, J.Am.Chem.Soc., *66*, 1707 (1944).

Example 4

Preparation of 6 - (α - aminophenylacetamido) - penicillanic acid

(a) To an ice-cooled solution of 0.01 mole of 6 - N - trimethylsilylaminopenicillanic acid trimethylsilyl ester and 1.30 g. (0.01 mole) of redistilled quinoline in 25 ml. of dry tetrahydrofurane was added with stirring 2.06 g. (0.01 mole) of α-aminophenylacetylchloride hydrochloride. The reaction flask was cooled in an ice-bath and the reaction mixture protected from moisture. After the addition was complete the stirring was continued for 15 minutes in the ice-bath and then for further 60 minutes at room temperature.

100 ml. of water and 30 ml. of a normal potassium bicarbonate solution were then added and the stirring continued for another 5 minutes. The neutral solution was washed twice with ether, and then analyzed by paper chromatography to show the presence of the penicillin together with a small amount of 6-aminopenicillanic acid. The solution was worked up by conventional methods to give 2.27 g. (65.3%) of the penicillin as a white crystalline powder with a purity of 80% as determined by alkalimetric assay. The product inhibited the growth of *Staph. aureus Oxford* at a concentration of 0.03 mcg./ml.

The α - aminophenylacetylchloride hydrochloride was prepared by reacting D, L-α-aminophenylacetic acid with phosphorous pentachloride in acetylchloride for 20 hours. The product was isolated by filtration and after washing thoroughly with petroleum ether a white product was obtained which sintered above 100°C., but showed no precise melting point. The product contained 34.2% chlorine (Calc. for $C_8H_9Cl_2NO$: 34.41% Cl).

(b) By using a twofold excess of N-trimethylsilylaminopenicillanic acid trimethylsilyl ester to the amount of α-aminophenylacetylchloride hydrochloride and omitting the organic base but otherwise performing the same operation as described above in (a) there was obtained 2.14 g. (74%) calculated on the used amount of α-aminophenylacetylchloride hydrochloride 1.7 g. (0.0085 mole). The product obtained was identical with the one in (a).

(c) *From 4-phenyloxazolid-2:6-dione*

A solution of 5.85 g. (0.033 mole) of 4-phenyl - oxazolid - 2:5 - dione in 25 ml. of dry dimethylformamide was added with stirring to a solution of 0.03 mol. of 6-N-trimethylsilylaminopenicillanic acid trimethylsilylester in 25 ml. of dry dimethylformamide, containing 3.54 g. (0.033 mole) of dry redistilled 2,6-lutidine. The reaction was performed under strictly anhydrous conditions and the reaction flask was cooled to −40°C. The addition was completed in 20 minutes and afterwards the mixture was stirred for three hours. 5 ml. of water and 50 ml. of ether were then added and the precipitate formed isolated by filtration. After washing and drying 7.9 g. of a slightly yellow powder was obtained, which by paper chromatography and microbiological assay was shown to contain 30% of the penicillin. This corresponds to a total yield of 22%. The 4 - phenyl - oxazolid-2:5 - dione was prepared according to Leuchs and Geiger Ber. *41*, 1722 (1908).

Example 5

Preparation of 6 - (D - α - aminophenylacetamido) - penicillanic acid

From 43.3 g. (0.2 mole) of dried 6-aminopenicillanic acid and 87.8 g. (0.6 mole) of N-trimethylsilyl diethylamine 0.2 mole of 6-N - trimethylsilylaminopenicillanic acid trimethylsilyl ester was prepared as previously described.

It was dissolved in 250 ml. of dry tetrahydrofurane together with 33 g. (0.254 mole) of redistilled quinoline and chilled in ice. 55.5 g. (0.27 mole) of D - α - aminophenylacetic acid chloride hydrochloride was then added with stirring, in portions during 10 minutes. During the addition the temperature rose to about 10°C. After stirring for further 40 minutes at room temperature the resulting almost clear solution was poured into 2 litres of water, with efficient stirring. The mixture was neutralised with 2 N sodium hydroxide, filtered and washed twice with ether in order to remove the quinoline.

After freeze-drying 77.2 g. of a light brown powder with a purity of 52% (hydroxylamine assay) was obtained. This corresponds to a corrected yield of 57% of the penicillin.

Paper chromatography showed that the product contained the desired penicillin as the only antibiotic compound. The penicillin was subsequently obtained with a purity of 85.2% (alkalimetric assay). This product had an optical rotation of $[\alpha]_D^{23}$ — +257° (phosphate buffer pH 7.0) and inhibited the growth of *Staph. aureus Oxford* at a concentration of 0.01 mcg./ml.

The D - α - aminophenylacetylchloride hydrochloride used in this example was prepared from the D - amino - phenylacetic acid and phosphorcus pentachloride. It was obtained as a colourless labile compound con-

taining 32.5% Cl (Calc. for $C_9H_9Cl_2NO$: 34,41% Cl). According to its infrared spectrum it probably contained some α-aminophenylacetic acid hydrochloride as an impurity.

EXAMPLE 6

Preparation of 6 - (α - amino - α - phenylpropionamido) - penicillanic acid

To an ice cold solution of 0.22 mole of 6-N - trimethylsilylaminopenicillanic acid trimethylsilyl ester and 46.5 g. (0.36 mole) of redistilled quinoline in 250 ml. of dry tetrahydrofurane 59.1 g. (0.268 mole) of DL - α-methyl - α - aminophenylacetylchloride hydrochloride was added with stirring during 10 minutes. During the addition the temperature rose to about 20°C. and was held there for a further 35 minutes. Care was taken to exclude moisture from the reaction mixture. The almost clear solution was poured into 2 l. of water and neutralised with 2 N sodium hydroxide, filtered and washed twice with ether. There was obtained 2.53 l. solution with a penicillin content of 25.1 mg./ml., which corresponds to 63.5 g. of the penicillin i.e. a yield of 80%. The penicillin was isolated with a purity of 73.2% (alkalimetric assay) and inhibited the growth of *Staph. aureus Oxford* at a concentration of 0.63 mcg./ml.

The acid chloride was prepared by reacting the α - methyl - α - aminophenylacetic acid with phosphorous pentachloride. It was obtained as a white powder with a Cl content of 30.5% ($C_9H_{11}Cl_2NO$ requires 32,22% Cl). According to its IR-spectrum it contained some α - methyl - α - aminophenylacetic acid hydrochloride as an impurity.

EXAMPLE 7

By using the known chloride hydrochlorides of glycine and DL-leucine the corresponding penicillins were prepared as described in Example 6.

	Yield	Purity	MIC*
α-aminomethylpenicillin	55%	65%	0.63
α-amino-γ-methylbutyl-penicillin	45%	67%	0.25

* Minimum inhibitory concentration against Staph. aureus Oxford in mcg./ml.

EXAMPLE 8

Preparation of 6 - (D - α - amino - γ - methylvaleramido) - penicillanic acid

6-aminopenicillanic acid (2.2 g.) was heated with N - trimethylsilyl - diethylamine (5.8 g.) for 45 minutes at 80°C., while the formed diethylamine was removed continuously by distillation at a slightly reduced pressure. The excess of the N - trimethylsilyldiethylamine was removed *in vacuo*, leaving the trimethylsilyl derivative of 6-aminopenicillanic acid as a viscous residue, which was dissolved in 40 ml. of dry ether. To this solution quinoline (1.55 g.) was added, followed by D-leucyl chloride hydrochloride (2.4 g.), while stirring and cooling in an ice bath. After 45 minutes the reaction mixture was poured into 150 ml. of water, neutralized with dilute sodium hydroxide and washed well with ether. By paper chromatography the aqueous phase was found to contain 1.6 g. (49%) of a 6 - (D - α-amino - γ - methylvaleramido) - penicillanic acid together with 0.6 g. of unreacted 6-aminopenicillanic acid. The penicillin was isolated by concentrating the water solution *in vacuo* at 20°C., after adjusting the pH to 5.0. Reprecipitation from water gave 1.15 g. with a purity of 69.8% (alcalimetric assay). By paper chromatography it was shown that the product was free from 6-aminopenicillanic acid.

This substance was found to inhibit the growth of *Staph. aureus Oxford*, at a concentration of 0.25 mcg./ml. and to contain in its IR-spectrum a strong band at 1770 cm^{-1}, indicating the presence of a β-lactam ring.

The D-leucyl chloride hydrochloride (Found: Cl 35.9; $C_6H_{13}Cl_2NO$ requires Cl, 38.11%) was prepared by reacting D-leucine with an equivalent amount of phosphorus pentachloride in carbon tetrachloride.

EXAMPLE 9

Preparation of 6 - (D - α - amino - β-phenylpropionamido) - penicillanic acid

6-aminopenicillanic acid (3.1 g.) and N-trimethylsilyldimethylamine (10 g.) were heated for 15 minutes at 60°C. while a slow stream of nitrogen was passed through the reaction flask in order to remove the dimethylamine as it was formed. The excess of the N - trimethylsilyldimethylamine was distilled off *in vacuo* leaving the trimethylsilyl derivative of 6-aminopenicillanic acid as a viscous residue, which was dissolved in 25 ml. of dry benzene. N,N-dimethylaniline (1.6 g.) was added to the solution followed by D-phenylalanyl chloride hydrochloride (3.0 g.) in portions during 5 minutes, while stirring and cooling in an ice bath. After stirring for one hour the almost clear reaction mixture was

poured into 50 ml. of water, neutralised by the addition of 2 N sodium hydroxide and washed well with ether. By paper chromatography the aqueous phase was found to contain 3.1 g. (86%) of 6 - (D - α - amino - β-phenylpropionamido) - penicillanic acid together with a small amount of unreacted 6-aminopenicillanic acid. After acidifying to pH 5.0 the water solution was concentrated *in vacuo* at 20°C., whereby 1.1 g. of the penicillin with a purity of 89% (hydroxylamine assay) was precipitated.

This substance was found to inhibit the growth of *Staph. aureus Oxford*, at a concentration of 0.13 mcg./ml. and to contain in its IR-spectrum a strong band at 1780 cm^{-1} showing the presence of a β-lactam ring.

The D-phenylalanyl chloride hydrochloride (Found: Cl 30.2; $C_9H_{11}Cl_2NO$ requires Cl 32.2%), was prepared by shaking D-phenylalanine with one equivalent of phosphorus pentachloride in methylene chloride. It probably contained some hydrochloride of the phenylalanine as an impurity (weak IR-absorption at 1720 cm^{-1}).

EXAMPLE 10

Preparation of 6 - (α - amino - Σ - methylheptanamido) - penicillanic acid

6-aminopenicillanic acid (4.3 g., 0.02 mole) was heated together with N - trimethylsilyldiethylamine (14.5 g.) for 45 minutes at 80°C., while the formed diethylamine was continuously removed by applying a slight suction to the reaction flask. The excess of the N - trimethylsilyl - diethylamine was distilled off *in vacuo* leaving the trimethylsilyl derivative of the 6-aminopenicillanic acid as a residue, which was dissolved in 50 ml. of dry benzene. N,N-dimethylaniline (2.4 g., 0.02 mole) was added followed by a solution of α - amino - Σ - methylheptanoyl chloride hydrochloride (0.02 mole) in 25 ml. of methylene chloride, while stirring and cooling in an ice bath. After 90 minutes the reaction mixture was poured into 100 ml. of water, neutralised by the addition of 2 N sodium hydroxide and washed with ether. By paper chromatography the aqueous phase was found to contain 3.3 g. (46%) of the 6 - (α - amino-Σ - methylheptanamido) - penicillanic acid together with some unreacted 6-aminopenicillanic acid. The pH was adjusted to 5.0 and the aqueous phase was concentrated *in vacuo* at room temperature to give 1.4 g. of the penicillin as a white precipitate. The product had a purity of 63% (hydroxylamine assay) and contained about 10% of 6-aminopenicillanic acid as an impurity.

This product was found to inhibit the growth of *Staph. aureus Oxford* at a concentration of 0.63 mcg./ml. and to contain in its IR-spectrum a strong band at 1776 cm^{-1}, showing the presence of a β-lactam ring.

The α - amino - Σ -methylheptanoyl chloride hydrochloride used in this example was prepared by reacting α - amino - Σ-methylheptanoic acid (3.2 g., 0.02 mole) with an equivalent amount of phosphorus pentachloride in methylene chloride till a clear solution was obtained. The solvent was distilled off *in vacuo* at 20°C. in order to remove the formed phosphorus oxychloride, and the residue was redissolved in methylene chloride and used directly. The IR-spectrum of this solution showed a strong band at 1770 cm^{-1} attributable to an acid chloride moiety.

EXAMPLE 11

Preparation of 6 - (α - amino - β - methyl-β - phenylbutyramido) penicillanic acid

6-Aminopenicillanic acid (1.5 g.) was heated for 45 minutes at 80°C. with N-trimethylsilyl diethylamine (7.0 g.) while dry nitrogen was passed through the reaction flask to remove the diethylamine as it was formed. The excess of the N-trimethylsilyl-diethylamine was distilled off *in vacuo* and the residue dissolved in 25 ml. of dry benzene. N,N - dimethylaniline (0.83 g.) was added followed by α - amino - β - methyl - β - phenylbutyryl chloride hydrochloride (2.2 g.), with stirring and cooling in an ice bath. After 1 hour the reaction mixture was poured into 50 ml. of water, neutralized and washed with ether. By paper chromatography the aqueous phase was found to contain as the only antibiotic substance 1.9 g. (70%) of the 6 - (α - amino - β-methyl - β - phenylbutyramido) - penicillanic acid, which after acidification to pH 5.0 with dilute hydrochloride acid, was isolated by concentrating the solution *in vacuo* at 20°C. There was obtained 0.8 g. with a purity of 59% (hydroxylamine).

This substance was found to inhibit the growth of *Staph. aureus Oxford*, at a concentration of 6.25 mcg./ml. and to contain in its IR-spectrum a strong band at 1776 cm^{-1} showing the presence of a β-lactam ring.

The α - amino - β - methyl - β - phenylbutyryl chloride hydrochloride (Found: Cl 25.9; $C_{11}H_{15}Cl_2NO$ requires Cl, 28.5%) was prepared by reacting the α - amino - β-methyl - β - phenylbutyric acid with an equivalent amount of phosphorus pentachloride in methylene chloride. It was probably contaminated by some hydrochloride of the amino acid (weak IR-absorption at 1720 cm^{-1}).

Using the method outlined in the examples 8 to 11 the following amino-penicillins were also prepared:

	Acylation yield	Purity	MIC
6-(D-α-aminopropionamido)-penicillanic acid	43	51	2.5
6-(L-α-aminopropionamido)-penicillanic acid	41	38	1.25
6-(D-α-amino-β-methylbutyramido)-penicillanic acid	35	44	2.5
6-(L-α-amino-β-methylbutyramido)-penicillanic acid	27	42	25
6-(L-α-amino-γ-methylvaleramido)-penicillanic acid	34	39	2.5
6-(L-α-,Σ-diaminovaleramido)-penicillanic acid	30	46	12.5
6-(α-amino-β-phenylpropionamido)-penicillanic acid	83	50	0.63
6-(L-α-amino-β-phenylpropionamido)-penicillanic acid	53	51	2.5

In the table are given: The acylation yield, as determined by paper chromatography of the reaction solution, the purity of the isolated product as determined by the hydroxylamine method and the minimum inhibitory concentration (MIC) in mcg./ml. of the product against *Staph. aureus Oxford*.

EXAMPLE 12

Preparation of 6 - (α - amino - γ - methylmercapto - butyramido) - penicillanic acid

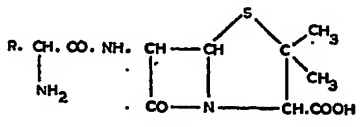
N - (2 - hydroxy - 1 - naphthal) - DL-methionine (3.0 g.) was dissolved in 25 ml. of anhydrous dimethylformamide together with dry triethylamine (2.0 g.). To this solution chloroformic acid ethyl ester (2.2 g.) was added with stirring at −10°C. and after 15 minutes a solution, in 25 ml. of anhydrous ether, of the trimethylsilyl derivative of 6-aminopenicillanic acid, prepared as described in example 8 from 6-aminopenicillanic acid (2.2 g.) and N - trimethylsilyl - diethylamine (7 g.). Immediately after the addition the temperature in the reaction mixture was raised to 25°C. and held there for one hour. Water (75 ml.) was added and the mixture was acidified to pH 2 and chilled in an ice bath for 30 minutes, while continuing the stirring. The mixture was neutralized and washed well with ether. By paper chromatography the obtained aqueous solution was found to contain 0.9 g. (25%) of 6 - (α-amino - γ - methylmercaptobutyramido)-penicillanic acid together with an equal amount of unreacted 6-aminopenicillanic acid. After acidification to pH 5.0 the penicillin was precipitated by concentrating the solution *in vacuo* at 20°C. There was obtained 0.5 g. of the penicillin with a purity of 41% (hydroxylamine assay). The product contained traces of 6-aminopenicillanic acid.

This product was found to inhibit the growth of *Staph. aureus Oxford*, at a concentration of 0.63 mcg./ml. and to contain in its IR-spectrum a strong band at 1770 cm^{-1}, showing the presence of a β-lactam ring.

The N - (2 - hydroxy - 1 - naphthal) - DL-methionine, m.p. 161.5—163.0°C. (Found: C, 63.6; H, 6.12; N, 4.62; S, 10.5. $C_{16}H_{17}NO_3S$ requires: C, 63.34; H, 5.65; N, 4.62; S, 10.57%) was prepared by shaking DL-methionine with a 25% excess of 2-hydroxy-1-naphthaldehyde in dimethylformamide till a clear solution was obtained. The solvent was removed *in vacuo* and the residue triturated with dry benzene.

WHAT WE CLAIM IS:—

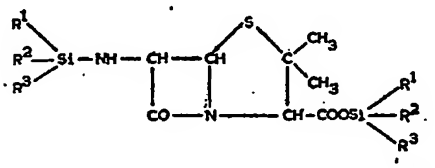
1. A process for the preparation of penicillins of the general formula:



(I)

and non-toxic salts thereof, where R is a hydrogen atom or an alkyl, aryl or aralkyl

group which may be substituted, which process comprises reacting a silyl derivative of 6-aminopenicillanic acid of the general formula:



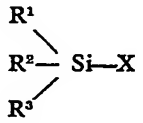
(II)

where R^1, R^2 and R^3 are the same or different and each is an alkyl, aryl or aralkyl group, or a solution thereof, with a reactive derivative of a carboxylic acid of the general formula:

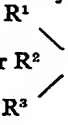
$$\begin{array}{l} R.\,CH.\,COOH \\ | \\ NH_2 \end{array} \qquad \text{(III)}$$

in which the amino group is protected, and thereafter removing the silyl groups by hydrolysis or alcoholysis.

2. A process as claimed in claim 1, wherein there is employed as the silyl derivative of 6-aminopenicillanic acid the crude reaction mixtures obtained when 6-aminopenicillanic acid is reacted with silylating agents of the general formula:



(IV)

wherein R^1, R^2 and R^3 are as defined in claim 1 and X is halogen or the group $N<^{R^4}_{R^5}$ or $S—R^5$ where R^4 is hydrogen or R^1, and R^5 is hydrogen, R^1 or R^2  Si, or solutions of such mixtures.

3. A process as claimed in claim 1 or claim 2, wherein the reactive derivative of the carboxylic acid is an activated ester, acid chloride or bromide, acid anhydride or mixed anhydride with another carboxylic, sulphonic or inorganic acid, or a derivative obtained from the carboxylic acid and a carbodiimide or an N,N^1-carbonyldiimidazole.

4. A process as claimed in any one of claims 1 to 3 wherein the reaction is carried out in the presence of an organic tertiary base.

5. A process as claimed in any one of claims 1 to 3, wherein the reaction is carried out in an excess of the silyl compound.

6. A process as claimed in any one of the preceding claims, wherein a trialkylsilyl derivative of 6-aminopenicillanic acid is reacted with a cyclic anhydride of an amino acid, or with an amino acid chloride hydrochloride.

7. A process for the preparation of penicillins as claimed in claim 1 or claim 2 substantially as described with reference to any one of Examples 4 to 12 hereinbefore set forth.

8. A penicillin when prepared by a process claimed in any one of claims 1 to 7.

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